Application No. 09/972,268 Declaration under 37 CFR 1.131

Application No:

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

09/972,268

Applicants:

Peter R. Baum, William C. Fanslow III, Timothy E. Lofton,

Eric A. Sorensen, and Adel Youakim

Filed:

October 5, 2001

Title:

NECTIN POLYPEPTIDES

TC/Art Unit:

1644

Examiner:

Maher M. Haddad

Docket No.:

3101-A

Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.131

We, Peter R. Baum, William C. Fanslow III, Timothy E. Lofton, Eric A. Sorensen, and Adel Youakim, the undersigned, hereby declare that:

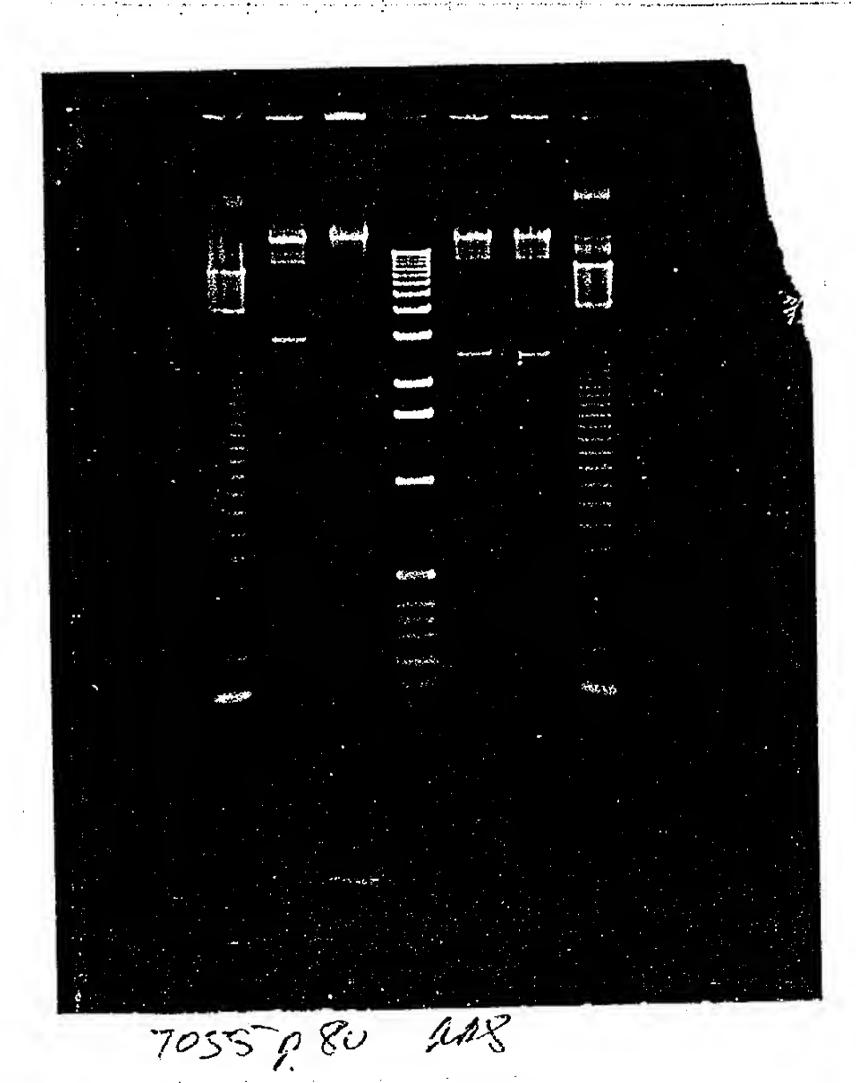
- This Declaration is made by the inventors of the above-captioned patent application in order to establish a date of invention in the United States prior to April 1, 2000.
- Prior to April 1, 2000, a DNA clone that encodes human nectin-3 polypeptide (also called "B7L4" polypeptide) had been isolated and its sequence determined in the United States by inventors named in the subject application, as evidenced by the Exhibits A and B enclosed herewith. The works described in Exhibits A and B were completed in this country prior to April 1, 2000.
- 3. Exhibit A is a copy of a page from one of the laboratory notebooks of Eric A. Sorensen, written in his handwriting, describing a restriction enzyme digest of an isolated lambda phage clone called "HuB7L4 11-1". All dates on the copy have been redacted.

- 4. Exhibit B (eight pages) is a copy of a computer printout that is incorporated into one of the laboratory notebooks of Eric A. Sorensen, showing the results of the sequencing of the HuB7L4 11-1 clone insert that was performed at the direction of Eric A. Sorensen. The amino acid sequence shown below the corresponding nucleotide sequences is that of human nectin-3 as presented in SEQ ID NO:2 of the above-captioned application (and is identical to amino acids 8 through 549 of SEQ ID NOs 4 and 6). The first page of Exhibit B indicates the location of a predicted signal sequence cleavage site, and the fourth page of Exhibit B indicates the location of the start of the transmembrane domain. All dates on the copy have been redacted.
- 5. Therefore, on a date prior to April 1, 2000, the inventors of the above-captioned application had determined the amino acid sequence of a human nectin-3 polypeptide including the extracellular domain of a mature form of human nectin-3.
- 6. As a person signing below: I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

	Date:
Peter R. Baum	
Milliam 6. Tanslow III	Date: June 23, 2005
William G. Fanslow III	
Timothy E. Vofton	Date: 23 JUNE 2003
Timothy E. Lofton Jan X. Journal	Date: June 24, 2003
Eric A. Sorensen Adel Youakim	Date: June 24, 2003

Book No.

m Page No. 7	man a la grande de la compansión de la comp			e Lean of the second se	Salar and Administrative methods	ng (195m) (1955) ayan marang da			· · · · · · · · · · · · · · · · · · ·				er per transmission in agricultural			e semi	
	ODN	n	for	HUB	724	1-2	125	11-	1	a- 1	13	(fr	om	FR	1.5.	414	
	ODN sut;		PEG	fo.	- t4	3	we	hs	\mathcal{I}	was	54	Vu	eat:)			
	5,	10:	1 01	1+			Wasi	le E	1×	y	70%	Etc	#.		1 2	ec'd	A A A A A A A A A A A A A A A A A A A
	y out	he	t.	Ros	Uspe	-610	۷	o/n		<i>)</i> –	60	H ₂	0.			1	
		· ·					:	:		:			• : • : • :		ر تسدو تفضيعه	Angeria a marina da m Marina da marina da mari	eren (f. 16. m.) T
	Drees	, ,	Ø	DNAS	·	Ec	521	(NER	742	1, 509	ther)	and o	1 No	12 (.	NEB	boller	34
	J	1.)	ØD	NA	11-)	/	7 E	6521									1
				1,									4)	0	NH	e primerone e ser estamble (1 cum).	Comment of the state of the sta
		3.)	Ø DI	VA	13	y	E	is 12)					1-5	1 10	+ 51	fler	1 1
		Ÿ.)					1	1.11-2					- 5	1 0	MZyn	~e	*
								**************************************	; ;	;			9	λ	420		·
				-	1									37 02	- (50-90	
			<u>,</u>		1			· · · · · · · · · · · · · · · · · · ·		mer a dissort of the second second	***************************************				er sammer er self er selvenskare. V	The same of the sa	AND THE STATE OF STAT
A CONTRACTOR OF THE PROPERTY O	The second secon		£	// - /	((e)	9.	5.5	110	1/m		† † † † † † † † † † † † † † † † † † †			er men en men men men en e	And the second second	gue de la
and the second s	aming to the second of the sec	i di i i i i i i i i i i i i i i i i i	# 1	3	(6		ئى	7.7	210	last			engine in				
The second secon	The second secon	n , man in the physical in the	ener () व () कार स्वत्यक्त विस् े				- .,	o Terror e					alter comments of a			· · · · · · · · · · · · · · · · · · ·	



RESULTS:

Arcording to this get, the close #13 is way small compare to what I estimated by quelione & PCh I svess I'll re whit the DNH sign The Esas Fragment into 185

To Page No. 0

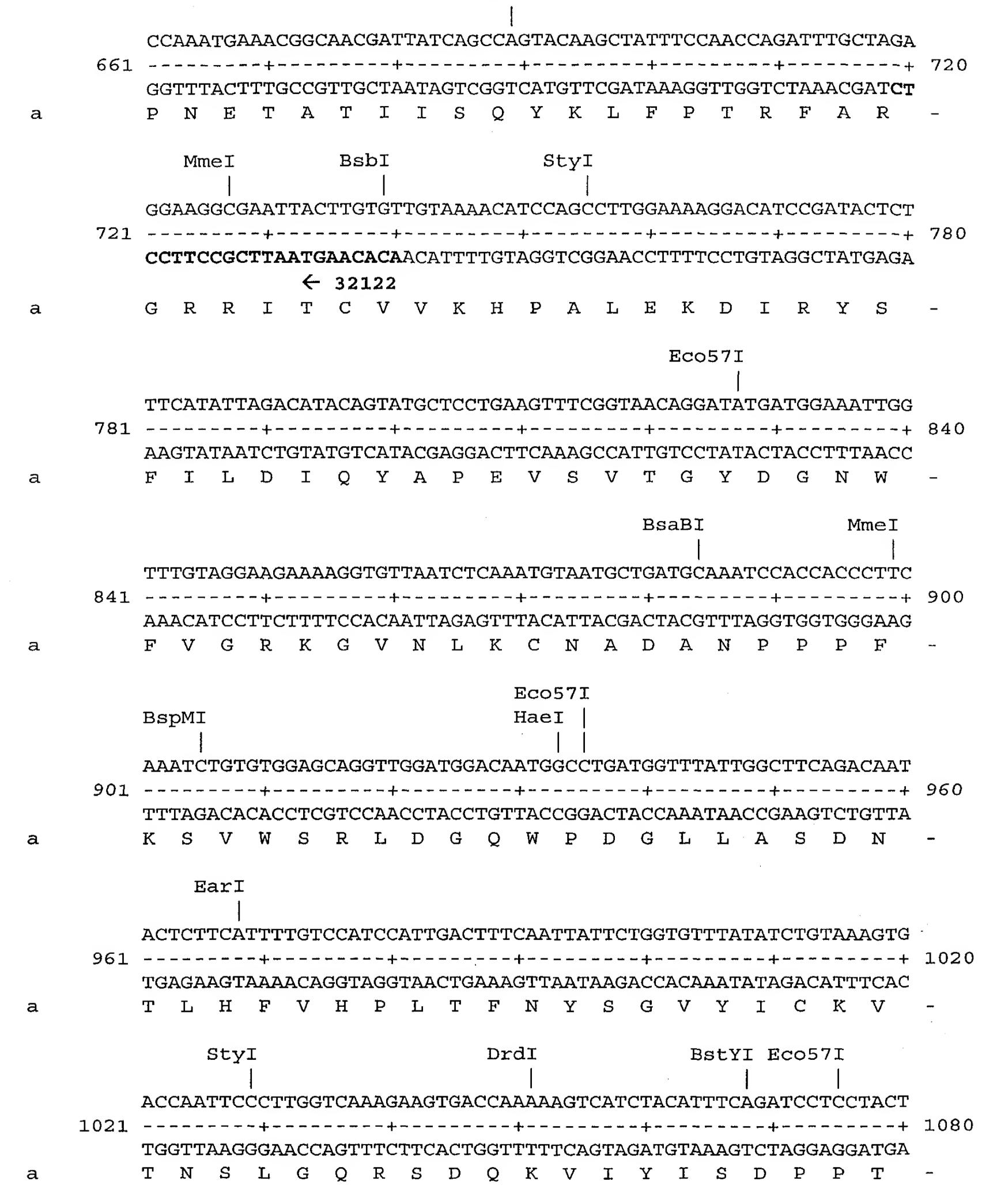
Vitnessed & Understood by me,	Date	Invented by	Dațe
Son Munit		Recorded by Jimmy	

```
(Linear) (MinSite=6) MAP of: hub714-11.seq check: 8088 from: 1 to: 3187
HuB7L4 #11 from KB library clone #11-1. Phage DNA: NOT CONFIRMED
sr6527 R. Sorensen
/bertlesj/sorensen/sr6527/hub7l4-11.seq
8139,8140,DPC#9117-20,12233-34,12759-60,12801
HuB7L4-11
With 164 enzymes: *
                  MspA1I
                 BsiEI
                EaeI
                EagI
               GdiII
         ApoI
               NotI
         ECORI
                          MspA1I
                                                      BpmI
 34356-->SalI-GCGGCCATGGCCCGGACCCCCGGCCC-
        V-D -A G M A R
                                                       34053 →
        CAAAAGAATTCGCGGCCGCTGTCCCCGCTGTGTCCTGGAGGCGCAAAGCACAACTTTCC
        \tt GTTTTCTTAAGCGCCGGCGACAGGGCGACACAGGACCTCCGCCGTTTCGTGTTGAAAGG
                          S P L C P G G K A Q L S
a
                                             BsaXI
                          BanII
                                            Hin4I
                       Bsp1286I
                                PstI
                                         BsaHI
                      Hin4I
                               SfcI BplI
    BseRI
                                                 MspA1I
        TCCGCTTCTCCTCGGAGCCGGGCTCCTGCTGCAGCCCCCGACGCCACCTCCGCTGCTG
        AGGCGAAGAGAGGAGCCTCGGCCCGAGGACGACGTCGGGGGCTGCGGTGGAGGCGACGAC
        SASLLGAGLLQPP
a
               MspA1I
                EarI
                SapI
                             EarI
                                     Bpu10I
               BpmI
                           SapI AlwNI BanI BspGI
                            (AgeI)
                   34359 --> ACCGGT-AGGCTCTGTGGTGCCTTAGCTGG 33684 →
        CTGCTGCTCTTCCCGCTGCTGCTCTTCTCCAGGCTCTGTGGTGCCTTAGCTGGACCAATT
        GACGACGAGAAGGGCGACGACGAGAAGAGGTCCGAGACACCACGGAATCGACCTGGTTAA
                                              ← 34032 (AP1 rev)
       LLLFPLLLFSRLCGALAGPI
a
      Predicted signal seq. Cleavage ^
                  NspI
             AflIII
           BspLU111
                                              MslI
        ATTGTGGAGCCACATGTCACAGCAGTATGGGGAAAGAATGTTTCATTAAAGTGTTTAATT
    181 -----+----+ 240
```

7-7.85 0 \$

		TA	ACA	CCI	'CGG	FTG1	ACA	GTG	TCG	STCA	TAC	CCC	TTT	CTT	'ACA	AAC	TAA	ΓTT	CAC	AAA	AATT	1
										\leftarrow	34	054										
a		I	V	E	P	Н	V	Т	A	V	W	G	K	N	V	S	L	K	С	L	I	-
							86															
	241	GA	AGT	'AAA	TGA	AAC	CAI	'AAC	ACA	GAI	TTC	ATG	GGZ	GAA	GAI	'ACA	TGG	CAA	AAG	TTC	ACAG	
	241.	CT	TCA	. TTT	'ACI	TTG	GTA	TTC	TGI	CTA	AAG	TAC	CCI	'CTI	'CTA	TGT	'ACC	GTT:	TTC	AAG	TGTC	
æ		E	V	N	E	T	I	${f T}$	Q	I	S	M	E	K	I	H	G	K	S	S	Q	
								37 -	T			7A T	_ T					T1				
								AC	mI 			AT	οI					Ear	 T			
		AC	TGT	TGC	'AGI	TCA	CCA	ATCC	CCA	ATA	TGG	ATT	CTC	TGT	'TCA	AGG	AGA	ATA	TCA	.GGG	AAGA	
	301				-+-			+	. ~			+			-+-			+ ~ -		- ~ -	+	360
				336		AAGT	'GG'1	:AGG	iGG'1	.''I'A'I	'ACC	T'AA	.GAG	ACA	'AG.T	"TCC	TCT	"1'A'1	'AGT	CCC	TTCT	
a a		${f T}$	v	A	V	Н	Н	P	Q	Y	G	F	S	V	Q	G	E	Y	Q	G	R	_
				Dra	I I																	
		GT	CTT	'GTT	ι ΊΤΑΑ	AAA	TTA	CTC	'ACT	'TAA	TGA	TGC	AAC	TAA!	'TAC	TCT	'GCA	AAT.	CAT	AGG.	ATTC	
	361			-	-+-			+	· 			+			-+-			+			+	420
			GAA											_							TAAG	
i.		V	اسلم	F	K	N	Y	S	L	N	D	A	${f T}$	I	T	L	H	N	1	G	F	_
																			Bm	rI		
		m.c		mma	maa	רי אי אי	יא רדו יא	CI N III	i CimiCi	1 /1 717171	7 00	mam	m * ^	la mai	aaa	aam	maa	7A 7A 7A	ന്ദ്ര		amaa	
	421	TC							_												GTCC +	480
		AG.	ACT			TTT 368		'GTA	.GAC	GTT:	TCG	ACA	ATG	TAA	.GGG	CGA	ACC	TTT	ACG	GGT	CAGG	
à.		S	D	s	G		Y	I	C	K	A	V	${f T}$	F	P	L	G	N	A	Q	S	<u></u>
															ما مادء	~		* ~ ~	~~~	.		
	481	TC	TAC	AAC	TGT -+-	'AAC	T'G'I'	'GT'T' +	'AGT' 												TTCT +	540
	101	AG.	ATG	TTG	ACA	TTG	ACA	CAA	TCA			•						•			AAGA	
ì		S	\mathbf{T}	\mathbf{T}	V	T	V	L	V	E	P	\mathbf{T}	V	S	L	I	K	G	P	D.	S	-
																	Alw	NI				
																'	4 de 18 a				3212	23 -
		TT.	TAA	TGA	TGG	AGG	AAA	TGA	AAC	AGT	AGC	AGC	CAT	TTG	CAT	CGC	AGC	CAC	TGG.	AAA	ACCC	
	541	70.70	 mm7\	~ ~ ~	-+-	 maa		+ תיים אי	~			_			•		 TCC	~ - +	 7\CC	— ~ ~	+ r GGG	600
a.		AA L	I	ACI.	ACC G	G	N	E	T	V	A	A	I	AAC C	I	ace A	A	T T	G	K	P	-
						Bm	rI															
			121 TGC	_	ጥልጥ	тGА	- CTG	GGA	AG G	тса	ጥርጥ	ፐርር	ፐርኔ	ገ ል ጥ	GGA	ΑΤС	ĊAC'	TAC.	AAC'	ԱՄ	TTTT	
	601				-+-			+				+			-+-			-			-	660
		CA	ACG	TGT				CCT	TCC	ACT	AGA	ACC.	ACT	TTA	CCT	TAG	GTG.	ATG	TTG.	AAG	AAAA	
		* *	24.	TT	←	330		ינו	~		т	~	T-1	1. #	1773	C	m	т	п	S.	ਾਂ	_
L		V	A	H	1	D	W	${f E}$	\mathbf{G}	D	T	G	\mathbf{E}	M	\mathbf{E}	S	Ŧ	T	7	Ü	Ľ	_ _

TatI



. . .

.

	ACTA	CC7.	عاططلا	יחיני א	aaa	ነጥ አ උ	י א אי	יין אין דיין דיי	CTTC	יממי	\ mac	ירייייר	ነአ አረ	·тc:С	ייייני ז	. רי א יו	יככז	 \cc7	י מזיכיר	F7 7A
1081		CCA																		ra -+ 1:
1001	TGAT	'GGT'	GGGA							-		•				•				. –
	T		_	Q	P	T		Q		H					D	I	_	D	L	
										Hin	ıcII									
						***	.~~	· ~ · · ·	.~~	4 % PY4PY	10000			. ~ ~ ~		1 78			~_ ~	
1141	GCAA	CAG																•		4C -+ 12
T T # T	CGTT		•										-							•
	A 7	'E	P		K				P		S		L	Ā		I		D	D	_
															S	cal				
					Taq						E	anI	I		Tat	I				
M	unI		Bs	rDI	(Bg	12)				Bs	:p12	861		Ear	I,	, 1,				
			~~~~		~					.~~	15300	~~~					~ .	· · · · · · · · · · · · · · · · · · ·		·
1001	ACAA	.T.T.G(JCAC	GAT	CAT	TGC	TAC	:TG1	AGT	r G G	:TGG	ODDD:	TCT	CTT	CAT -	AGT				
1201	TGTT	~ ~ ~ ~ ·	~ +		~ ~ ~ ~~~	7.00	~ ~ ~	יה כיה	TC7		' ' TA C' C		ጉተ- አርአ		~ C:T! N	— — + ПСЛ				+ 12
	TGTI						_	321			ACC	CCG	, ,	.CAA	GIA	. I CA	. I GF		71 7 (-FL
tart T								<i>3 </i>	<i>4</i> 7											
COLU I	T I							V	V	G	G	A	L	F	Ι	v	L	V	s	_
	<u>. </u>		_	_	_		_	•	-	_	_					•		•		
							Bs	p24	I											
			s	spI		Sfc		p24	ı 		Bbs	I						Bsp	241	- -
			S	spI 		Sfc		p24	I		Bbs	I 						Bsp)24I 	.
	GTTI	TGG(1			I 	-				į	GTT	TCG	TGG	AGA	.CTA		1	
1261	GTTT	TGG(AAT	ATT:	CTG	I CTA	TAG	 GAG	AAG	ACG	 GAC						CTI	 TGC:	
1261	GTTT CAAA		CTGG +- SACC	AAT 	ATT: TAA:	CTG + GAC	I CTA GAT	TAG 'ATC	GAG	AAG + TTC	ACG TGC	 GAC CTG	-+- CAA	 A GC	 ACC	+ TCT	 GAI	CTI 	TGC 	C + 13
1261	CAAA		CTGG +- SACC	AAT	ATT: TAA:	CTG + GAC	I CTA GAT	TAG 'ATC	GAG	AAG + TTC	ACG TGC	 GAC CTG	-+- CAA		 ACC	+ TCT	 GAI	CTI 	TGC 	C + 13
1261	CAAA V I	ACCO A	CTGG +- SACC G	AAT TTA' I	ATT TAA F	CTG + GAC C	I CTA GAT Y	TAG 'ATC R	 GAG CTC R	AAG + TTC R	ACG TGC R	GAC CTG T	-+- CAA F	 A GC R	acc G	+ TCT D	 GAT Y	CTI GAA F	TGC ACG A	C + 13 ;G -
	CAAA V I	ACCO A	CTGG +- SACC G	AAT TTA' I	ATT TAA F	CTG + GAC C	I CTA GAT Y	TAG 'ATC R	GAG CTC R	AAG + TTC R AAA	ACG TGC R	 GAC CTG T ATC	-+- CAA F ACA	 A GC R AAT	acc G AGA	+ TCT D	GAI Y TCI	CTI GAA F	TGC ACG AACA	C + 13 •G -
1321	CAAA V I	ACTA	CTGG +- SACC G ACAT	AAT	ATT TAA F ACC	CTG + GAC C ATC	I CTA GAT Y	TAG 'ATC R	GAG CTC R	AAG + TTC R AAA +	ACG TGC R	GAC T ATC	-+- CAA F ACA	 A GC R AAT	ACC G AGA	+ TCT D TGT	GAI Y TCI	CTI GAA F	TGC ACG	C + 13 G - A + 13
1321	CAAA V I	ACTA	CTGG +- SACC G ACAT	AAT	ATT TAA F ACC	CTG + GAC C ATC	I CTA GAT Y	TAG 'ATC R	GAG CTC R	AAG + TTC R AAA +	ACG TGC R	GAC T ATC	-+- CAA F ACA	 A GC R AAT	ACC G AGA	+ TCT D TGT	GAI Y TCI	CTI GAA F	TGC ACG	C + 13 G - A + 13
1321	CAAA V I AAGA TTCI	ACCA ACTA TGAT	CTGG +- SACC G +- GTA	AAT TTA' I TCC AGG'	ATT TAA F ACC.	CTG + GAC C ATC + TAG	I CTA GAT Y AGA 	TAT 'ATC' 'ATA'	GAG CTC R GCA	AAA + TTC AAA +	ACG TGC R AGA 	GAC CTG T TAG	-+- CAA F ACA -+- TGT	 AGC R AAT 	acc G AGA 	+ TCT D TGT + ACA	GAT Y TCT 	CTI GAA F TCA	TGC ACA	C + 13 G - A + 13
1321	CAAA V I	ACCA ACTA TGAT	CTGG +- SACC G +- GTA	AAT	ATT TAA F ACC.	CTG + GAC C ATC	I CTA GAT Y AGA 	TAG 'ATC R	GAG CTC R GCA	AAA + TTC AAA +	ACG TGC R AGA 	GAC CTG T TAG	-+- CAA F ACA -+- TGT	 A GC R AAT	acc G AGA 	+ TCT D TGT + ACA	GAT Y TCT 	CTI GAA F	TGC ACG	C + 13 G - A + 13
1321	CAAA V I AAGA TTCI	ACCO A ACTA TGAT	CTGG +- SACC G ACAT +- TGTA	AAT TTA' I TCC AGG'	ATT TAA F ACC. TGG'	CTG + GAC C ATC + TAG	I CTA GAT Y AGA TCT	TAG ATC R TAT 	GAG CTC R GCA CGT	AAG + TTC R AAA + TTT	ACG TGC R AGA 	GAC TT TAG	-+- CAA F ACA -+- TGT	 AGC R AAT TTA	ACC G AGA TCT	+ TCT D TGT + ACA	GAI Y TCI AGA	CTI GAA F TCA AGI	TGC ACA ACA TGT	C + 13 G -
1321	CAAA V I AAGA TTCI L125 K N GATG	ACCO A ACTA TGAT	CTGG +- SACC G ACAT +- TGTA	AAT TTA' TCC AGG'	ATT TAA F ACC. TGG'	CTG + GAC C ATC + TAG	I CTA GAT Y AGA D	TATATATATA	GAG GAG CTC R GCA CGT	AAA + TTT R AAA + TTTT	ACG TGC R AGA TCT	GAC TT TAG AGA	-+- CAA F ACA -+- TGT Q	 AGC R AAT TTA	ACC G AGA TCT	+ TCT D TGT + ACA V	GAT Y TCT AGA AGT	CTI GAA F TCA AGI	TGC ACA ACA TGT	C + 13 G -
1321 (32	CAAA V I AAGA TTCI L125 K N GATG	ACTA ACTA TGAT AGCTA	CTGG + - EACC G + - IGTA I TGA	AATA TTA' TCCA AGG'	ATT TAA F ACC. TGG'	CTG+ GAC+ TAG CCC	I CTA GAT Y AGA D	TATATATATATATATA	GAG CTC R CGT CGT	AAA + TTT AAA + K	AGA TCT E	GAC TT TAG AGA AGA	-+- CAA F ACA -+- TGT Q AAA -+-	AGC R AAT TTA CAA	ACC G AGA TCT AAA	+ TCT D TGT + ACA V	GAT Y TCT AGA AGT	CTI GAA F 'GAA AGI Q	TGC ACA ACA TGT	C + 13 G - A + 13 T -
1321 (32	CAAA V I AAGA TTCT LL 125 # K N GATG	ACTA ACTA TGAT AGCTA TGAT TGAT	CTGG + - EACC G + - IGTA I TGA	AATA TTA' TCCA AGG'	ATT TAA F ACC. TGG'	CTG+ GAC+ TAG CCC	I CTA GAT Y AGA D	TATATATATATATATA	GAG CTC R CGT CGT	AAA + TTT R AAA + TTTT K	AGA TCT E	GAC TTGTAG	-+- CAA F ACA -+- TGT Q AAA -+-	AGC R AAT TTA CAA	ACC G AGA TCT AAA	+ TCT D TGT + ACA V	GAT Y TCT AGA AGT	CTI GAA F 'GAA AGI Q	TGC ACA ACA TGT	C + 13 G - A + 13 T -
1321 (32	CAAA V I AAGA TTCT 125 K N GATG	ACTA ACTA TGAT AGCTA TGAT TGAT	CTGG+- EACC G CTA+- CTA+- ACT	AAT TTA' I TCC AGG' AAGA	ATT TAA F ACC. TGG' P TTA	CTG+ GAC C ATC+ TAG CCC+ GGG	I CTA GAT Y AGA TCT	TATA ATA M CAG	GAG CTC R CGT Q	AAA + TTT R AAA + TTTT K	ACG TGC R AGA TCT E	GAC TTGTAG	-+- CAA F ACA -+- TGT Q AAA -+- TTT	AGC R AAT TTA I CAA GTT	AGA TCT	TGT ACA TCC AGG	GAT Y TCT AGA AGT TCA	CTT CAAGT CAAGT	TGC ACA ACA CAA CAA CAA CAA CAA CAA	C + 13 G - A + 13 T -
1321 (32	CAAA V I AAGA TTCT L125 K N GATG CTAC D E	ACTA ACTA TGAT AGCTA TGAT TGAT	CTGG+- EACC G CTA+- CTA+- ACT	AATA TTA' I TCCA AGG' AGG' AAGA	ATT TAA F ACC. TGG' P TTA	CTG+ GAC C ATC+ TAG C C C P	I CTA GAT Y AGA TCT	TATA ATA ATA ATA ATA ATA ATA ATA ATA AT	GAG CTC R CGT Q	AAA + TTT R AAA + TTTT K	ACG TGC R AGA TCT E	GAC TTGTAG	-+- CAA F ACA -+- TGT Q AAA -+- TTT	AGC R AAT TTA I CAA GTT	AGA TCT	TGT ACA TCC AGG	GAT Y TCT AGA AGT TCA	CTT CAAGT CAAGT	TGC ACA ACA CAA CAA CAA CAA CAA CAA	C + 13 G - A + 13 T -
1321 (32	CAAA V I AAGA TTCT 125 K N GATG CTAC D E	ACTA ACTA TGAT TGAT TCGAT TCGAT	CTGG+- EACC G CTA+- CTA+- ACT	AATA TTA' I TCCA AGG' AGG' AGG' E	ATT TAA F ACC. TGG' P TTAC AATC	CTG + GAC C ATC + TAG S CCC + GGG	I CTA GAT Y AGA TCT	TATA ATA ATA ATA ATA ATA ATA ATA ATA AT	GAG CTC R CGT Q	AAA + TTT R AAA + TTTT K	ACG TGC R AGA TCT E	GAC TTGTAG	-+- CAA F ACA -+- TGT Q AAA -+- TTT	AGC R AAT TTA I CAA GTT	AGA TCT	TGT ACA TCC AGG	GAT Y TCT AGA AGT TCA	CTT CAAGT CAAGT	TGC ACA ACA CAA CAA CAA CAA CAA CAA	C + 13 G - A + 13 T -
1321 (32	CAAA V I AAGA TTCT L125 K N GATG CTAC D E	ACTA ACTA TGAT TGAT TCGA TCGA TCGA TCGA	CTGG+- EACC G ACAT+- TGTA I TGA+- ACT D	AATA TTA' I TCCA AGG' AGG' AGG' S	ATT TAA F ACC TGG' P TTAC AATC Y	CTG + GAC C ATC + TAG S CCC + GGG	I CTA GAT Y AGA TCT D	TATATATATATATATATATATATATATATATATATATA	GAG CTC R GCA CGT Q TGT ACA V	AAA + TTT R AAA + TTT K	ACG TGC R AGA TCT E	GAC TT ATC TAG AGA TCT E	-+- CAA F ACA -+- TGT Q AAA -+- TTT	AGC R AAT TTA CAA GTT K	ACC G AGA TCT AAA TTT	TGT ACA TCC AGG P	GAT Y TCT AGA AGT TCA TCA	CTT GAA F CTA AGT CTT	TGC ACA ACA CAA CAA CAA CAA CAA CAA CAA C	C + 13 G - A + 13 T + 14 A
1321 ~ 32	CAAA V I AAGA TTCT 125 K N GATG CTAC D E	ACTA ACTA TGAT TGAT TCGA TCGA TCGA TCGA	CTGG+- EACC G ACAT+- TGTA I TGA+- ACT D	AATA TTA' I TCCA AGG' AGG' AGG' S	ATT TAA F ACC TGG' P TTAC AATC Y	CTG + GAC C ATC + TAG S CCC + GGG	I CTA GAT Y AGA TCT D	TATATATATATATATATATATATATATATATATATATA	GAG CTC R GCA CGT Q TGT ACA V	AAA + TTT R AAA + TTT K	ACG TGC R AGA TCT E	GAC TT ATC TAG AGA TCT E	-+- CAA F ACA -+- TGT Q AAA -+- TTT	AGC R AAT TTA CAA GTT K	ACC G AGA TCT AAA TTT	TGT ACA TCC AGG P	GAT Y TCT AGA AGT TCA TCA	CTT GAA F CTA AGT CTT	TGC ACA ACA CAA CAA CAA CAA CAA CAA CAA C	C + 13 A + 13 T + 14 A -
1321 (32	CAAA V I AAGA TTCT L125 K N GATG CTAC D E	ACCO ACTA TGAT TGAT TCGA TCGA TCGA TCGA	TGG CTGG CAT CAT ITGA TGA D	AAT AAT TTA' I TCC AGG' AGG' AAGA S AGA AGA AGA	ATT TAA FACC TGG' PTAA AATA Y	CTG+ GAC C ATC+ TAG S CCC+ GGG P	I CTA GAT Y AGA TCT D	TAT ATA M CAG GTC S	GCA CGT ACA CGC CGC	AAA TTT KAAA TTT KAAA TTT K	AAA TTT AAA	GAC TT ATC TAG AGA TCT E	-+- CAA F ACA -+- TGT Q AAA -+- TTT N TCA -+-	AGC R AAT TTA I CAA GTG GTG	AGA TTT TTT N GAA	TGT TCT TGT ACA V TCC AGG P CAA+	GAT Y TCT AGA AGT TCA TCA TCA	CTI CAA F CTA AGI CTI N AGA AGA	TGC ACA ACA CAA CAA CAA CAA CAA CAA CAA C	C + 13 A + 13 T + 14 A - 15

 $oldsymbol{\cdot}$

t.

BglII BstYI 1501 a MslI NspI AflIII MslI BspLU11I DrdII 1561 a BsrGI TatI (NotI) GTAATTTCCAGGAGGGAGTGGTATGTTTAGCAACCACTGAATGTGACTTAACTATGTACA 1621 --CATTAAAGGTCCTCCCTCACCATACAAATCGTTGGTGACTTACACTGAATTGATACATGT <--34358 -CGCCGGCG S R R E W Y V * <--36018 a SpeI BclI SmlI ATGTTCATTCACACTAGTTGATCATTTTCAGATTGTTCATACTTTTTTCTTGAGGAAGAAT 1681 -----TACAAGTAAGTGTGATCAACTAGTAAAAGTCTAACAAGTATGAAAAAAGAACTCCTTCTTA HindIII Bce83I HindIII AAGCTTTTTCAAGTTGATTTTCAAGCTTACTTTTTTATATTCTAATCTGACAAATGAAAAT 1741 ------TTCGAAAAGTTCAACTAAAAGTTCGAATGAAAAATATAAGATTAGACTGTTTACTTTTA TatI Bce83I 1801 -----ApoI DraI Scal SwaI SmlI TatI TGTCTCAAGATTTAAATTTTAATGCAGAGTACTTTATTGGTGTGAGGCACACAGGTAAGA

1861 -----+----+ 1920

ACAGAGTTCTAAAATTAAAATTACGTCTCATGAAATAACCACACTCCGTGTGTCCATTCT

	HincII	ApoI	DraI										
	AGAAATGTCAACATTAAATGTATGACT		_										
1921	++++++												
	Bce83I	Tth111II SmlI	I ļ										
1981	ACCTTGACATTGTGTATTAAATGTTTAC		 CAAGTATGATGTTTGTT 	2040									
# 7	TGGAACTGTAACACATAATTTACAAATC	•	AGTTCATACTACAAACAA										
		BtsI HaeIV											
	ApoI	Hin4I	77 EIG 7 7 7 7 7 8 EIG 6 7 7 6 6 6 6 7 7 7 7 7 7 7 7 7 7 7 7										
2041	TAACATATACCTCTCAAAATTTATCACC		+	2100									
	SspI		SspI										
2101	AACTAATTCAAGAAATATTTATATATAT	TTTTTAATATACAA		2160									
21 .1. 0 .1.	TTGATTAAGTTCTTTATAAATATATATA	AAAAATTATATGTTI	TTTTATAAATCGGACTAC	2100									
	Tth	111II 											
2161	GAATGGCTTTCCTTTTCAAACATTATTT	-+	+ +	2220									
	MslI	MONI I CHAMONIAIC	ADDIARADALLIAGA										
	VspI Sf	CI 											
2221	CTGCATATTAATGAGCCTTGCCATAATTACTGTAGAGTGGCTTTTCAAAGATATTTTGTT+++ GACGTATAATTACTCGGAACGGTATTAATGACATCTCACCGAAAAGTTTCTATAAAACAA												
	GACGIAITACICGGAACGGIAIIAA	EarI	MAGITICIATAAAACAA										
		SapI 											
2281		-+		2340									
	CGTGATTTTGACACCATCATTTGAGTCA	•	TTCTCGTATTAATCGAC	·									
	SspI Bs GTCAATATTTTTGTCCAAAATACCTGCA	pMI AGAGTAATAAAATAC	ATACCTTTCDDDCDTGD										
2341		-+	+	2400									

	Tth111I	
	 TAATTATTAGTTTTTTTTTTCCTTTCTGGAACATGGATTTTGGTACATTAGCAGTAGCCT	
2401	ATTAATAATCAAAAAAAAAAGGAAAGACCTTGTACCTAAAACCATGTAATCGTCATCGGA	2460
2461	TATTTTAATGCTTTATGTCCTAAACATACTAATAGAAATGAAAAGACGCAGAGAGAG	2520
2521	SpeI ScaI TatI Eco57I SfcI ApoI TTCGGAATACTGAAGTACTAGTTTTAGAAATGAGACTTTCAGCCAACAATCTATAGAAAG+ AAGCCTTATGACTTCATGATCAAAATCTTTACTCTGAAAGTCGGTTGTTAGATATCTTTC	2580
2581	TatI AATTTTATGGACCATCTTGTTTTAGTTATTTAATGTTGATGTTGTTCAAATGGGTAAATG + TTAAAATACCTGGTAGAACAAAATCAATAAATTACAACTACAACAAGTTTACCCATTTAC	2640
2641	ApoI TACAGAAAGAAAATTTTAGAGTAAACTTGGAACTTTGGATATAACTAGAAAAAAACTAGAT + ATGTCTTTCTTTTAAAAATCTCATTTGAACCTTGAAACCTATATTGATCTTA BsmI	2700
2701	TATAGAATTAGTCGGTAACACTTGCTAATGGACATTGGCATTCATCTCCTTTTTCCTCCT+ ATATCTTAATCAGCCATTGTGAACGATTACCTGTAACCGTAAGTAGAGGAAAAAGGAGGA	2760
2761	AAGTGTATGTATGTGTTTTAAGATTTCTGTTTTTTACGATTAAAACTGGAAACATGAGGTT+ TTCACATACATACACAAAAATTCTAAAGACAAAAAATGCTAATTTTGACCTTTGTACTCCAA	2820
2821	TTTTGTTTTTTTTTACATAATTACATATATTCCTTCTGAATCATTTATCTTTTGAG++++++++++++++++++++++++++++++++	2880
	TthlllI SfcI	
2881	AAAGAAATGTTACCTAAACTTCAAATGTGCTTTTTTGTTGTGAGGTAATTAAATTGCTTC+ TTTCTTTACAATGGATTTGAAGTTTACACGAAAAAACAAAC	2940

•

2041			WWTIWIIGI				_ _ _					
2941	ATGTCACCT						+ 3000 CAAAAA					
3001	CTATTGCTA						GTTAGT + 3060					
3001	GATAACGAT'			_			,					
AGGAGAATCATAAATTAAATATTATTTTTTTTAATAAAAAGGCAAAGTAGTAGGTA												
3001	TCCTCTTAGTATTTAATTTATATAAAAACAATTATTTTTCCGTTTCATCATCCATGA											
						BsiE EaeI EagI GdiII NotI	Apol EcoRI I 					
Г	raI		Š	SspI		MspA1I						
	TTTAAACCC	rcccaacca	GCCCTTTCT	CAATATTCA'	TCAAATCTA	AAAC A GCGG	CCGCGA					
3121		+	-+	+	+		+ 3180					
	AAATTTGGG	AGGGTTGGT	CGGGAAAGA	GTTATAAGT	AGTTTAGAT	TTTGTCGCC	GGCGCT					
	ATTCAGC											
3181	35	187										
	TAAGTCG											
Enzymes	that do cu	ıt:										
AflIII	AloI	AlwNI	ApoI	BanI	BanII	BbsI	Bce83I					
BclI		BmrI	BplI	BpmI	Bpu10I	BsaAI	BsaBI					
BsaHI	_	BsbI	BseRI	BsiEI	BsmI	Bsp24I						
BspGI		BspMI	BsrDI	BsrGI	BstYI	BtsI	DraI					
DrdI	**	EaeI	EagI	EarI	Eco57I	EcoRI	GdiII					
HaeI		Hin4I	HincII	HindIII	MmeI	MslI	MspA1I					
MunI		NspI	PstI	SapI	Scal	SfcI	SmlI					
SnaBI		SspI	StyI	SwaI	TagII		Tth111II					
VspI	-	~ <u>~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ </u>				2002						
Enzymes t		cut:										
AarI		AccI	AceIII	AclI	AflII	AhdI	ApaI					
ApaLI		AvaI	AvrII	BaeI	BamHI	BbvCI	BcgI					
BciVI			Bpull02I	BsaI	BsaWI	BseSI	BsgI					
BsiHKAI		BspEI	BsrBI	BsrFI	BssHII	BssSI	BstAPI					
BstDSI		BstXI	BstZ17I	Bsu36I	ClaI	DraIII	EciI					
Eco47III		EcoO109I	EcoRV	FseI	FspI	HaeII						
HpaI		MluI	MscI	NarI	Ncol	NdeI	NgoAIV					
NheI	-	NsiI	NspV		Pfl1108I	PflMI	PinAI					
PmeI		PpiI	PshAI	Psp5II	PvuI	PvuII	RcaI					
RleAI		SacI	SacII	SalI	SanDI	SbfI	SexAI					
SfiI		SgrAI	SmaI	SphI		Sse8647I	StuI					
SunI	-	XbaI	XhoI	XmnI		. —						
				-3								

TACAGTGGAGGCTTACAAAATTATTGTGACAACTATTTTGAAGCTGAAAGGATAGTTTTT